Investigation of the absorption of granulocyte-colony stimulating factor following nasal administration with small and large microspheres in sheep

Materials and Methods

Materials

Granulocyte-colony stimulating factor (G-CSF) was supplied by Amgen as a 4 mg/ml solution in HCl, at pH 3.5.

L-α-lysophosphatidylglycerol (LPG) was purchased from Sigma (L 1756).

Starch microspheres (approximately 1280 mg) from Perstorp were used. SSMS - less than 10 μm dry diameter, SMS - 25μm dry diameter.

Ultra pure water ("Elgastat UHP", Elga, High Wycombe, Bucks., UK) was used throughout.

All other reagents were, at least, of standard laboratory grade.

Sheep

Sixteen (16) male cross-bred sheep of known weight were used in this study. The average weight of the sheep was 40 kg (S.E.M. = ±1.04). The sheep were normally housed outside, but, were brought inside for the duration of the study. The animals were not fasted prior to G-CSF administration. An in-dwelling Viggo secalon cannula was placed in one of the external jugular veins of each animal on the first day of the study and, whenever necessary, was kept patent by flushing it with heparinised saline solution. This cannula was removed upon the completion of the study and the sheep were returned to their normal housing.

Preparation of G-CSF formulations

For each of the powder formulations, the appropriate volume of a mannitol (0.6518%) and sorbitol (0.0724%) stabilising solution (S) was added to sufficient SMS or SSMS for 8 sheep of 40 kg (640 mg). The appropriate volume of the 4 mg/ml G-CSF solution and 12.8 ml of a 0.5% (5.0 mg/ml) LPG solution in water was added (Table 1). Sufficient water was also added to maintain the total volumes, as shown in Table 1. After mixing, these formulations were freeze-dried until free-flowing powders had been obtained. This process did not take longer than 24 hours. The final product was loaded into the administration devices and were stored overnight with desiccant at -20°C prior to use.

Table 1. Dose Schedule for G-CSF formulations with SMS (dry diameter of 25μm)

	Doses per kg					
Formulation Received and Route	G-CSF (μg)	LPG (mg)	SMS (mg)	Volume (ml)	Additives* (mg)	Weight (mg)
G-CSF/LPG/SMS, nasal	40	0.2	2.0	---	0.267	2.507
G-CSF/LPG/SMS, nasal	80	0.2	2.0	---	0.534	2.814

* *Mannitol/Sorbitol buffer used for the formulations.*

Table 2. Dose Schedule for G-CSF formulations with SSMS (dry diameter of less than 10μm

	Doses per kg .					
Formulation Received and Route	G-CSF (μg)	LPG (mg)	SSMS (mg)	Volume (ml)	Additives* (mg)	Weight (mg)
G-CSF/LPG/SSMS, nasal	40	0.2	2.0	---	0.267	2.507
G-CSF/LPG/SSMS, nasal	80	0.2	2.0	---	0.534	2.814

* *Mannitol/Sorbitol buffer used for the formulations.*

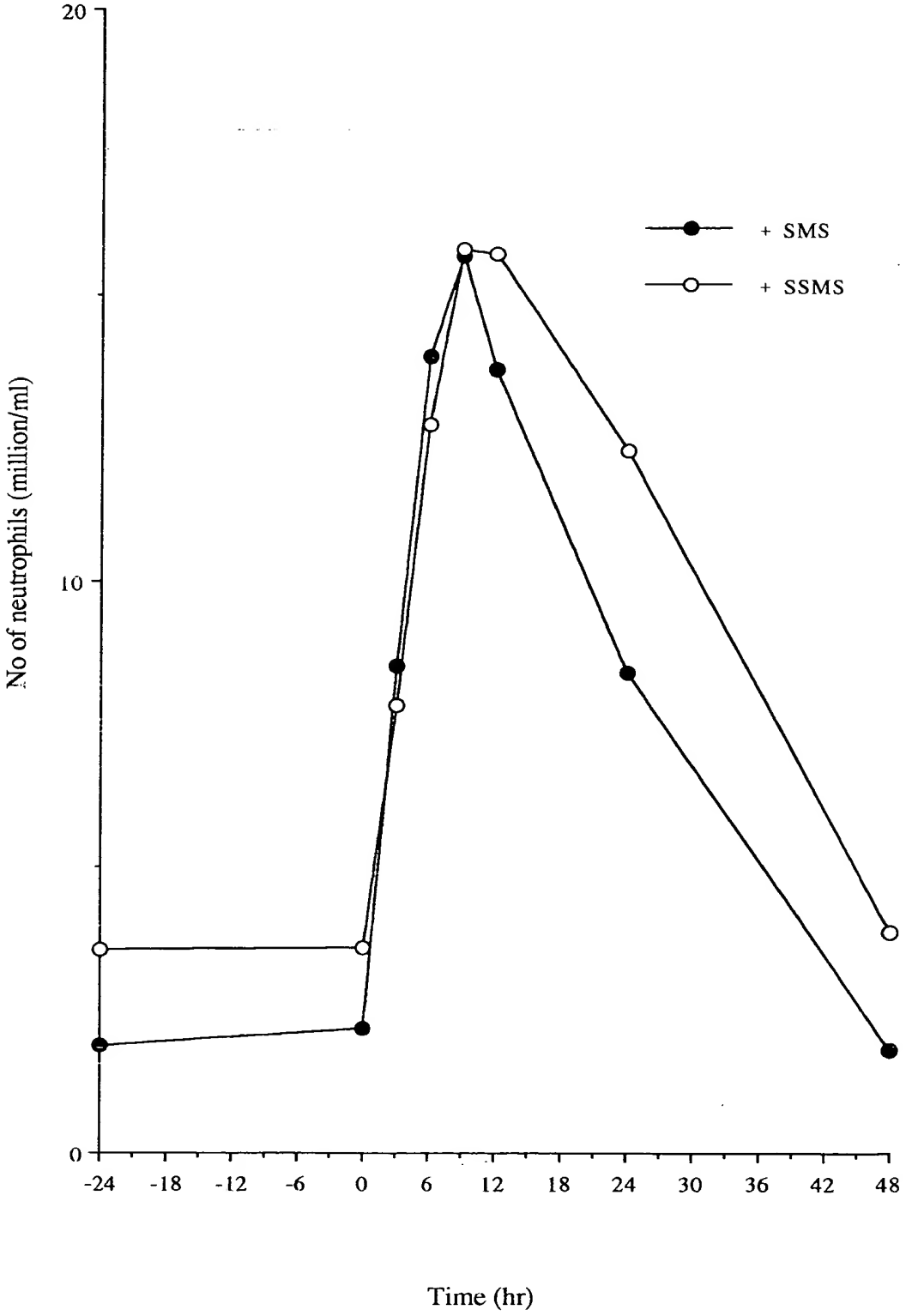
Administration of G-CSF formulations

For intranasal administration of the powders, a nasal tube was loaded with the formulation and then inserted into the nostril of the sheep before blowing the powder into the nasal cavity.

Results

The results are shown in the attached Figures.

Absolute counts of neutrophils following intranasal administration of G-CSF at 40µg/kg in combination with 0.2 mg/kg LPG and 2 mg/kg of either SMS or SSMS as powder formulation in sheep.



Absolute counts of neutrophils following intranasal administration of G-CSF at 80µg/kg in combination with 0.2 mg/kg LPG and 2 mg/kg of either SMS or SSMS as powder formulation in sheep.

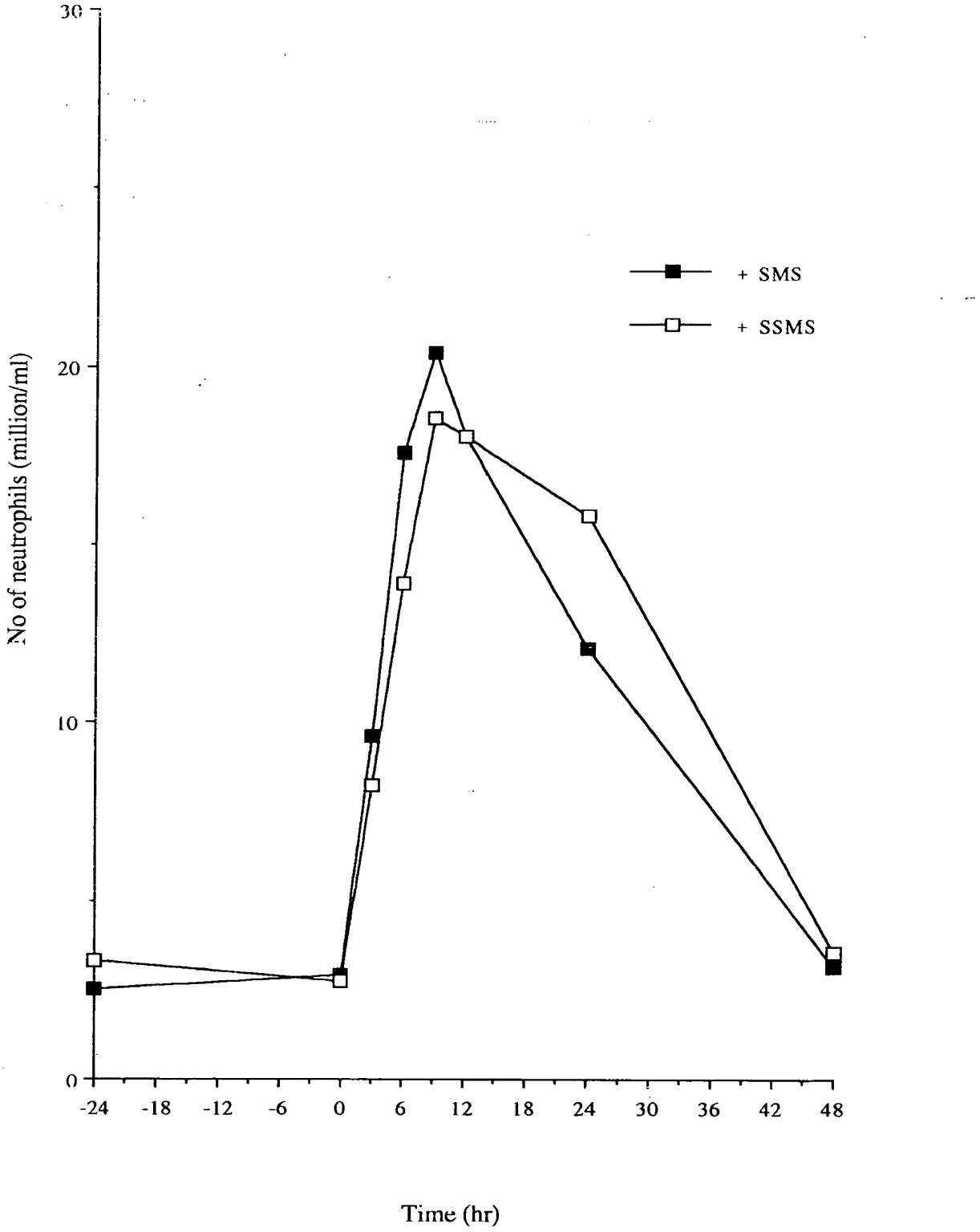


EXHIBIT 15

DECLARATION IN SUPPORT OF US PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: DANBIOSYST UK LIMITED
Serial No: 08/065,676
Filed: 21 MAY 1993
Title: SMALL PARTICLE DRUG COMPOSITIONS
Group Art Unit: 1502
Examiner: KISHORE, G

DECLARATION

1. I am Lisbeth Illum of 19 Cavendish Crescent North, The Park, Nottingham NG7 1BA, England. I am the inventor for the above application. I am managing director of Danbiosyst UK Ltd., assignee of the above application.

2. In order to investigate the absorption of granulocyte-colony stimulating factor following nasal administration with small and large microspheres, appropriate experiments were carried out under my instruction. Details of these experiments and the results obtained are given in the attached Exhibit A.

3. It can be seen from the results shown in the figures of Exhibit A that at both dosage levels of G-CSF, the AUC for the small microspheres (less than 10μm) is considerably larger than the large microspheres (25μm). This indicates that considerably greater absorption of G-CSF was achieved following administration with the small microspheres than with the large microspheres.

4. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed at Nottingham, England

This 2 day of March

US Serial No. 08/065,676

This is Exhibit A, referred to in

my Declaration, signed concurrently.

Lisbeth Illum

2 March 1994

Date